

## Supporting Information:

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**Table S1: Lipid A peak assignments from Fig. S1**

<b>Peak assignment</b>	[M-2H] <sup>2-</sup> Calculated <i>m/z</i>	KM225 <sup>a</sup> [M-2H] <sup>2-</sup> Observed <i>m/z</i>	KM199 [M-2H] <sup>2-</sup> Observed <i>m/z</i>	KM191 [M-2H] <sup>2-</sup> Observed <i>m/z</i>	KM192 [M-2H] <sup>2-</sup> Observed <i>m/z</i>
Kdo <sub>2</sub> -lipid A	1117.661	1117.63	1117.62	1117.59	1117.60
+ Na	1128.652	1128.62	1128.62	1128.57	1128.59
+ phosphate	1157.644	1157.61	1157.60	1157.56	1157.58
+ phosphate + Na	1168.635	1168.60	1168.59	1168.55	1168.57
+ phosphate + 2×Na	1179.626	1179.60	1179.61	1179.55	1179.57
+ pEtN	1179.165	N.D.	1179.12	N.D.	N.D.

<sup>a</sup>All strains were grown in LB medium as described. <sup>b</sup>N.D., not detected.

**Table S2: Lipid A peak assignments from Fig. S2**

<b>Strain</b> <b>Growth condition<sup>a</sup></b>	KM225		KM199		
	LB [M-2H] <sup>2-</sup> Calculated <i>m/z</i>	LB + Ca <sup>2+</sup> [M-2H] <sup>2-</sup> Observed <i>m/z</i>	LB [M-2H] <sup>2-</sup> Observed <i>m/z</i>	LB + Ca <sup>2+</sup> [M-2H] <sup>2-</sup> Observed <i>m/z</i>	
Kdo <sub>2</sub> -lipid A	1117.661	1117.63	1117.64	1117.59	1117.64
+ Na	1128.652	1128.62	1128.64	1128.57	1128.63
+ phosphate	1157.644	1157.61	N.D. <sup>b</sup>	1157.56	N.D.
+ phosphate + Na	1168.635	1168.60	N.D.	1168.55	N.D.
+ phosphate + 2×Na	1179.626	1179.60	N.D.	1179.55	N.D.
+ pEtN	1179.165	N.D.	1179.15	N.D.	1179.14
+ pEtN + Na	1190.156	N.D.	1190.14	N.D.	1190.13

<sup>a</sup>All strains were grown in LB medium +/- 5 mM Ca<sup>2+</sup> as described. <sup>b</sup>N.D., not detected.

**Table S3: Lipid A peak assignments from Fig. S3**

Strain Growth condition <sup>a</sup>	KM225		KM199	
	LB [M-4H] <sup>4-</sup> Calculated	LB + Ca <sup>2+</sup> [M-4H] <sup>4-</sup> Observed	LB [M-4H] <sup>4-</sup> Observed	LB + Ca <sup>2+</sup> [M-4H] <sup>4-</sup> Observed
Peak assignment	<i>m/z</i>	<i>m/z</i>	<i>m/z</i>	<i>m/z</i>
Kdo <sub>2</sub> -lipid A	558.307	558.32	558.33	558.31
+ phosphate	578.318	578.31	N.D. <sup>b</sup>	578.30
+ phosphate + Na	583.814	583.81	N.D.	583.80
+ pEtN	589.079	589.07	589.08	589.07
+ pEtN + Na	594.574	N.D.	594.58	N.D.

<sup>a</sup>All strains were grown in LB medium +/- 5 mM Ca<sup>2+</sup> as described. <sup>b</sup> N.D., not detected.

**Table S4.** Strains and plasmids used in this study

Strains or plasmids	Description	Reference or Source
<b>Strains</b>		
DH5 $\alpha$		
MG1655	Wild-type <i>E. coli</i>	
NM22540	MG1655 $\Delta$ araBAD, leu <sup>+</sup>	N. Majdalani
PM1205	mal::lacI <sup>q</sup> , araC <sup>+</sup> , P <sub>BAD</sub> -cat-sacB-lacZ, mini λ tet <sup>R</sup>	(Mandin & Gottesman, 2009)
PM1413	PM1205 lacI' <sup>q</sup> ::P <sub>BAD</sub> -rpoS-lacZ, arcZ::tet	(Mandin & Gottesman, 2010)
NM127	NM22540 mini λ(cm)	N. Majdalani
KM112	PM1205 mgrR-lacZ transcriptional fusion (positions -60 to +10 of mgrR promoter)	(Moon & Gottesman, 2009)
KM129	NM22540 ΔmgrR::kn	(Moon & Gottesman, 2009)
KM137	KM129 mini λ(tet)	(Court <i>et al.</i> , 2003)
KM161	NM22540 mini λ(tet)	(Court <i>et al.</i> , 2003)
KM176	NM22540 ΔeptB::cm	(Moon & Gottesman, 2009)
KM177	KM129 ΔeptB::cm, ΔmgrR::kn	(Moon & Gottesman, 2009)
WBB06	W3110 ΔwaaCF::tet	(Reynolds <i>et al.</i> , 2005)
KM186	WBB06 (pWaaCF)	This study
KM191	KM176 ΔwaaCF::tet	KM176 + P1(KM186)
KM192	KM177 ΔwaaCF::tet	KM177 + P1(KM186)

KM199	KM137 $\Delta waaCF::cm$	KM137 + waaCF-CmF + waaCF-CmR
KM201	MG1655 $\Delta mgrR::kn$	MG1655 + Ig957 KnF + Ig957 KnR
KM202-1	PM1205 <i>eptB-lacZ</i> transcriptional fusion (positions -60 to +94 of <i>eptB</i> promoter), Point mutation in -10 region (TACT → TAAT)	PM1205 + eptB-60upF +eptB_+95lacZR
KM202-3	PM1205 <i>eptB-lacZ</i> transcriptional fusion (positions -60 to +94 of <i>eptB</i> promoter), no mutation	PM1205 + eptB-60upF +eptB_+95lacZR
KM125	PM1205 <i>PBAD-</i> <i>eptB-lacZ</i> translational fusion (positions +1 to +133 <i>eptB</i> leader region and 9 amino acids of the coding region)	PM1205 + pBAD-eptBF eptB-1801lacZR
KM224	PM1205 <i>PBAD-</i> <i>eptB-lacZ</i> $\Delta mgrR::kn$	KM125 + P1(KM129)
KM225	NM22540 $\Delta waaCF::cm$	KM161 + waaCF-CmF + waaCF-CmR
KM233	PM1205 <i>PBAD-</i> <i>eptB-lacZ</i> translational fusion (positions +1 to +448 of <i>eptB</i> leader and coding regions)	PM1205 + pBADeptBF + eptB-+448lacZR
KM234	KM233 $\Delta mgrR::kn$	KM233 + P1 (KM129)
KM238	PM1205 <i>eptB-lacZ</i> transcriptional fusion (positions -150 to +94 of <i>eptB</i> promoter)	PM1205 + eptB-150upF + eptB_+95lacZR
KM250	PM1205 <i>eptB-lacZ</i> transcriptional fusion (positions -150 to +94 of <i>eptB</i> promoter), mutation on extended -10 (TG → AC)	PM1205 + ex-10eptBF + eptB-150upF +eptB_+95lacZR
KM259	PM1205 <i>eptB-lacZ</i> transcriptional fusion (positions -100 to +94 of <i>eptB</i> promoter)	PM1205+ eptB-1—upF + eptB_+95lacZR
KM260	PM1205 <i>eptB-lacZ</i> transcriptional fusion (positions -75 to +94 of <i>eptB</i> promoter)	PM1205 + eptB-75upF +eptB_+95lacZR
KM263	PM1205 <i>eptB-lacZ</i> transcriptional fusion (positions -60 to +50 of <i>eptB</i> promoter)	PM1205 + eptB-60upF + eptB_+95lacZR
KM278	PM1205 <i>eptB-lacZ</i> transcriptional fusion (positions -50 to +94 of <i>eptB</i> promoter)	PM1205 + eptB-50upF + eptB_+95lacZR
KM299	PM1205 <i>eptB-lacZ</i> transcriptional fusion (positions -150 to +94 of <i>eptB</i> promoter), mutation on -65 (GTTTA → CAAAT)	PM1205 + mut65 eptBF + mut65eptBR +

		eptB-150upF + eptB_+95lacZR
KM376	PM1205 <i>PBAD-</i> <i>eptB-lacZ</i> $\Delta$ <i>mgrR::kn</i> $\Delta$ <i>arcZ::tet</i>	KM224 + P1 (PM1413)
KM373	PM1205 <i>PBAD-</i> <i>eptB-lacZ</i> translational fusion (positions +1 to +133 of <i>eptB</i> leader region and 9 amino acids of the coding region) but has complementary mutation on G97C	PM1205 + eptBG97CF eptBG97CR + eptB-150upF +eptB_95lacZR
KM375	KM373 $\Delta$ <i>arcZ::tet</i>	KM373 + P1 (PM1413)
KM399	KM375 $\Delta$ <i>arcZ::tet</i> , $\Delta$ <i>mgrR::kn</i>	KM375 + P1 (KM129)
KM401	PM1205 <i>eptB-lacZ</i> transcriptional fusion (positions -50 to +50 of <i>eptB</i> promoter)	PM1205 + eptB-50upF + eptB_+50lacZR
KMT13010	PM1205 <i>rpoEP1::lacZ</i> transcriptional fusion (positions -86 to +1 of <i>rpoE</i> P1)	K. Thompson, Howard University; PM1205 + KT932 + KT933
KMT14034	PM1205 <i>rpoEP2::lacZ</i> transcriptional fusion (positions -95 to +1 of <i>rpoE</i> P2)	K. Thompson, Howard University; PM1205 + KT936 + KT937
<b>Plasmids</b>		
pTrc99A	Amp <sup>R</sup> ; Ptrc expression vector, pBR origin	(Guzman <i>et al.</i> , 1995)
pTrc99A-rpoE	Amp <sup>R</sup> ; <i>rpoE</i> coding region under Ptrc promoter in pTrc99A	K. Thompson
pWaaCF	Amp <sup>R</sup> ; <i>waaCF</i> coding region under Plac promoter in pWSK29	(Reynolds <i>et al.</i> , 2005, Majdalani <i>et al.</i> , 1998)
pBR-Plac	Amp <sup>R</sup> ; <i>lac</i> promoter-based expression vector having a pBR322 origin	(Guillier & Gottesman, 2006)
pArcZ	Amp <sup>R</sup> ; AatII-EcoRI <i>arcZ</i> -containing fragment cloned into the same sites in pBR-Plac	(Mandin & Gottesman, 2010)
pArcZ C70G	Amp <sup>R</sup> ; C70G site directed mutation in ArcZ in pArcZ	(Mandin & Gottesman,

		2010)
pSA508	Amp <sup>R</sup> ; a derivative of pBI24; in vitro transcription vector, pBR origin	(Choy & Adhya, 1993)
pSA508-eptB	Amp <sup>R</sup> ; position -150 to +94 of the <i>eptB</i> promoter region	This study
pSA508-rybB	Amp <sup>R</sup> ; position -100 to +70 of the <i>rybB</i> promoter region	This study

**Table S5. Oligonucleotide primers and probes in this study**

Oligo name	Sequence (5' to 3')
	Oligonucleotides used for cloning plasmids
rybB-100ecoRF	GGGCCGAATTCATGGTATGCCAGGATTAGG
rybB+70pstIR	GGCCTGCAGATCACCTGAAACCGAAATG
eptB-150cepRF	TGCATCAAACGCATCAACGAAATGTGAATTCAATGACTCGTAAAAT
eptB+94pstIR	CGATTGATGTATCTCATGCAAACCTGCAGTGGACAAACAGGT
	Oligonucleotides used for constructing various lacZ fusions
eptB-150upF	CGAAGCGGCATGCATTTACGTTGACACCATCGAATGGCGCAATGACTCGTAAAATTTGGG
eptB-100upF	CGAAGCGGCATGCATTTACGTTGACACCATCGAATGGCGCGTAACGCTAAAGTCTTTT

eptB-60upF	CGAAGCGGCATGCATTACGTTGACACCATCGAATGGCGCTTGTGCTTCATGCACACTC
eptB-75upF	CGAAGCGGCATGCATTACGTTGACACCATCGAATGGCGCTTGCATTTGTAAATTGTG
eptB-50upF	CGAAGCGGCATGCATTACGTTGACACCATCGAATGGCGC ATGCACACTCTTCCCCACA
eptB+50lacZR	TAACGCCAGGGTTTCCCAGTCACGACGTTGAAAACGACCATAGCTGTTCCGTGTGA CGGCAGGGAAAAAGTGCAGGA
eptB+25lacZR	TAACGCCAGGGTTTCCCAGTCACGACGTTGAAAACGACCATAGCTGTTCCGTGTGAGCTTAG TGGCTTTCAGGCC
eptB_+95lacZR	TAACGCCAGGGTTTCCCAGTCACGACGTTGAAAACGACCATAGCTGTTCCGTGTGATGGACA AACAGGTGATAACA
ex-10 eptBF	TTTTCCCTTGCTGACGTCTACTTATT CGCGCGTGT
ex-10 eptBR	CGCGAATAAGTAGACGT CAGCAAAGGGAAAAAGTGTG
mut65 eptBF	CAAAC TTGCATTTCATT TTTGTGCTTCATGCACACTCT
mut65 eptBR	TGCATGAAGCACAAAAAATGAAAAATGCAAGTTGAAAAG
mut 35 eptBF	TGCACACTCTTCCC GTGAGATT CCCTTGCTGTGGTCT
mut 35 eptBR	GGGAAAGAGTGTGCATGAAGTCTCACACAGCAAAGGGAAA
pBad-eptBF	ACCTGACGCTTTATCGCAACTCTCTACTGTTCTCCATGCGCGTGTAGATT TACTTA
eptB-180lacZR	GGCCAGGGTTTCCCAGTCACGACGTTGAAAACGACGGCGAAGGTACCCAGTACGGTGG
eptB250r	ATAGCTGGCACCTGCGGAAAAGAGCACCACCGAGCGATGCCAGAATACGCC
eptB+448lacZ	GGCCAGGGTTTCCCAGTCACGACGTTGAAAACGACGGCTCGGAGCAAGGTGTAGCGAC
deeplac	TAGGAGCGACCTTATGAGTCAGAATACG
placF	TGTGAGCGGATAACATTGACATTGTG
eptB-knF	ACTTGCATTTGTAAATTGTGCTTCATGCACACTCTTTAGAAAAACTCATCGAGCA
eptB-KnR	GCGAGAAAGTCAGCAGGCCGCTTAGTTAGCCGCTGCCTC AAAGCCACGTTGTGTCTCAA
eptBG97C F	ACCTGTTGTCCAGGCTTGTGATGAGATACTC
eptBG97C R	TCTCATGCAAACAAAGCCTGGACAAACAGGTGATAAC

waaF_CmF	CATGG CCTGGCTGAA TCGCGACGCA TAAGAGCTCT GCATGTGTGACGGAAGATCACTCG
waaC_CmR	ATGAATGAAGTTAAAGGATGTTAGCATGTTAACCTTAACCAGCAATAGACATAAGCG
Ig957KnF	CGAGTTAACGCTCCGTTAACATTGAGAAAAGTAAAGGCCACGTTGTCTCAA
Ig957KnR	ATGACGGCGAAAAAAACCGCCAGTAAACCGGGCGGTGAATTAGAAAAACTCATCGAGCA
KT932	<b>CGAAGCGGCATGCATTACGTTGACACCATCGAATGGCGCTTAGGTGGTGAAATAAAAGGCCG</b>
KT933	GGGTAACGCCAGGGTTTCCCAGTCACGACGTTGAAACGACGGCCAGTGAATCCGTAA TCATGGTcatagctgtttccctgtgaaatttgttatccgcataattccAGAATCTGAACATCGCATTATC
KT936	<b>CGAAGCGGCATGCATTACGTTGACACCATCGAATGGCGCAGTTATAATGATAGATAATGATCCG</b>
KT937	GGGTAACGCCAGGGTTTCCCAGTCACGACGTTGAAACGACGGCCAGTGAATCCGTAA TCATGGTcatagctgtttccctgtgaaatttgttatccgcataattccGCAAAGGGTTAGAGTGTCTCGTTTT
	Biotinylated probes used for Northern blots
eptB-bio	GTACGGTGGCGGCCAGTTCAACAAACAGCAGAAATG
ompA-bio	CCATTGTTGTTGATGAAACCAGTGTCACTGGTACTGGGACCAG
SsrA-bio	CGCCACTAACAAACTAGCCTGATTAAGTTAACGCTCA

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**Figure Legends: Supplemental Figures****Figure S1.** ESI-MS analysis of doubly-charged LPS from  $\Delta waaCF$  derivatives

The ESI mass spectra of the doubly-negatively charged LPS ions are shown for A) KM225 ( $\Delta waaCF$ ), B) KM199 ( $\Delta waaCF\Delta mgrR$ ), C) KM191 ( $\Delta waaCF\Delta eptB$ ), and D) KM192 ( $\Delta waaCF\Delta mgrR\Delta eptB$ ). All four strains were grown in LB medium. The structure of the LPS is shown in Fig. 1 and the peak assignments are in Table S1.

**Figure S2.** Effects of  $Ca^{2+}$  on the ESI-MS of quadruply-charged LPS from  $\Delta waaCF$  derivatives

The ESI mass spectra of the quadruply-negatively charged LPS ions are shown for A) and B) KM225 ( $\Delta waaCF$ ) and C) and D) KM199 ( $\Delta waaCF\Delta mgrR$ ). The two strains were grown in LB medium for A) and C), while B) and D) were grown in LB medium supplemented with 5 mM  $Ca^{2+}$ . The structure of the LPS is shown in Fig. 1 and the peak assignments are in Table S2.

**Figure S3.** ESI Effects of  $Ca^{2+}$  on the ESI-MS of doubly-charged LPS from  $\Delta waaCF$  derivatives

The ESI mass spectra of the doubly-negatively charged LPS ions are shown for A) and B) KM225 ( $\Delta waaCF$ ) and C) and D) KM199 ( $\Delta waaCF\Delta mgrR$ ). The two strains were grown in LB medium for A) and C), while B) and D) were grown in LB medium supplemented with 5 mM  $Ca^{2+}$ . The structure of the LPS is shown in Fig. 1 and the peak assignments are in Table S2.

**Figure S4.** Alignments for the *eptB* promoter and leader

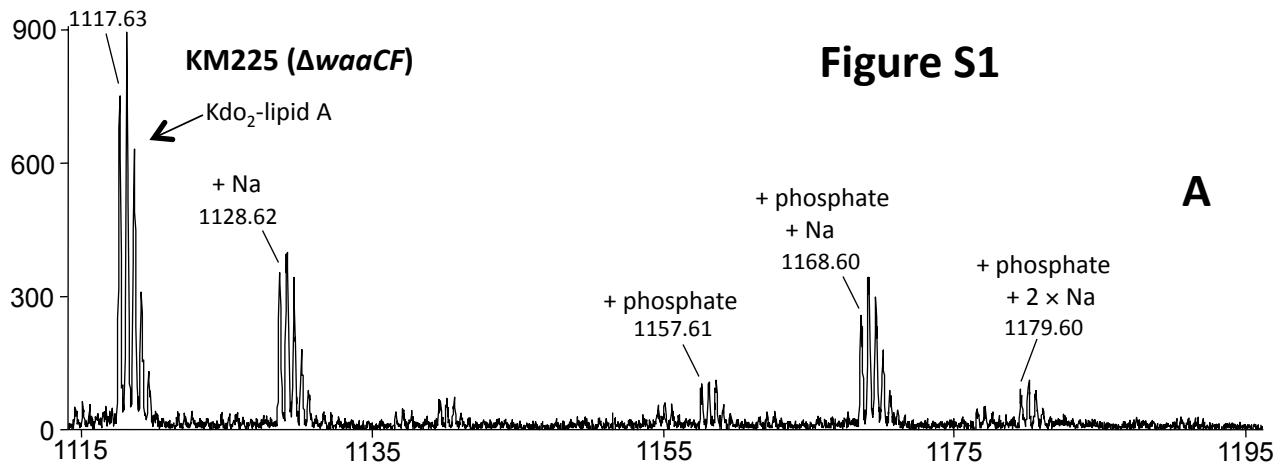
The region upstream of the *eptB* start codon in *E. coli* K12 is shown (EcK12). It was aligned using BLAST to microbial genomes, and selected sequences are shown for *Escherichia fergusonii* (Efer), *Salmonella enterica* (Salm), *Enterobacter cloacae* (EntCl) and *Klebsiella pneumoniae* (Kleb). The leader is considerably longer in *Salmonella enterica*; the extra sequence is shown. A conserved A/T rich upstream region is shaded in grey. The consensus sequence for Sigma E from Rhodius et al (Rhodius et al., 2006) is shown, with nucleotides conserved in the *eptB* promoter underlined. Spacing of 5-6 nucleotides between the -10 region and the starting nucleotide (G) is also a characteristic of these promoters; the *eptB* promoter has a spacing of 5 nucleotides. The -35 and -10 regions are highlighted in yellow, as is the ATG that begins the EptB ORF. The sequence of a portion of MgrR is shown above the region in the *eptB* mRNA with which it is predicted to pair. The region that pairs with ArcZ (Fig. 8) is highlighted in blue.

**Figure S5.**  $Ca^{2+}$  effects on sigma E promoters

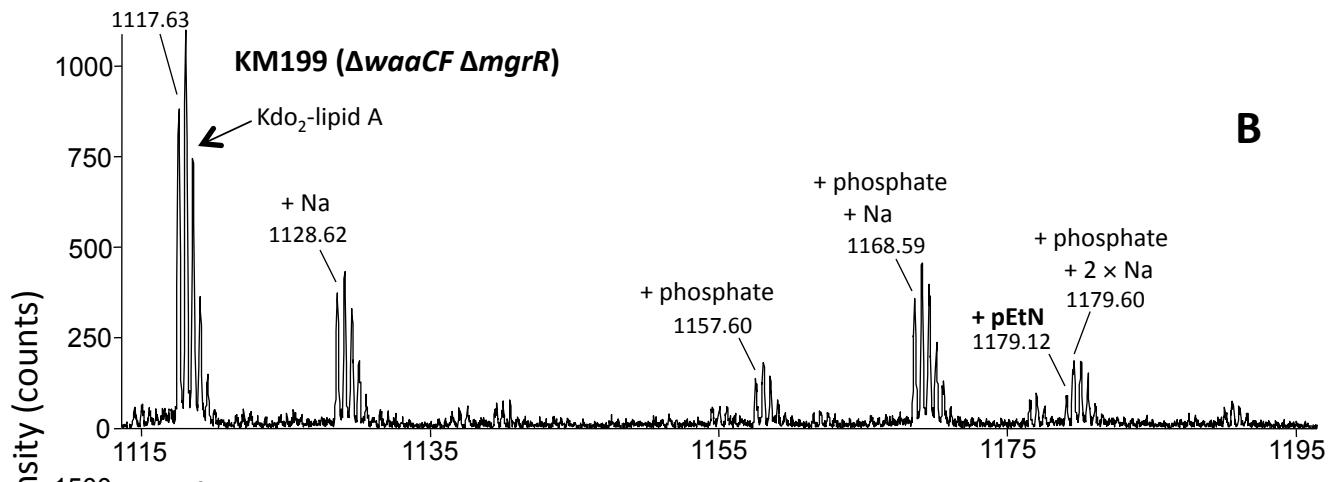
Transcriptional fusions to *rpoE* P1 (KMT13010) and P2 (KMT14034) were tested for the effect of  $CaCl_2$ . Cells were grown in LB at 37°C to exponential phase (O.D. 0.6) and split into two cultures, with (red lines) or without (black lines) 50 mM  $CaCl_2$ . Note that P2 is known to be Sigma E dependent, and thus if  $Ca^{2+}$  affected sigma E activity, an effect should be seen here.

**Figure S1**

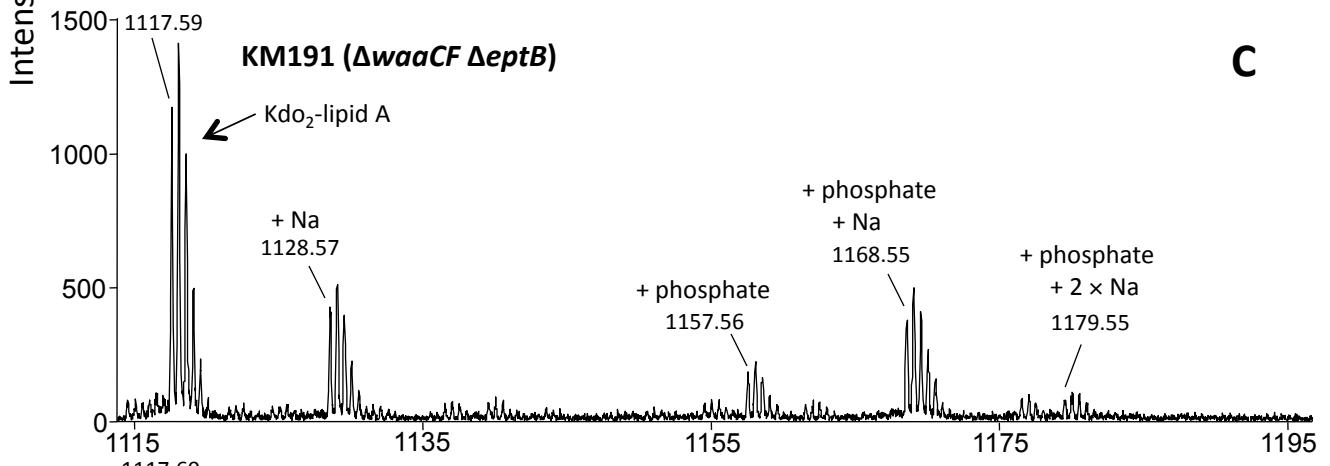
**A**



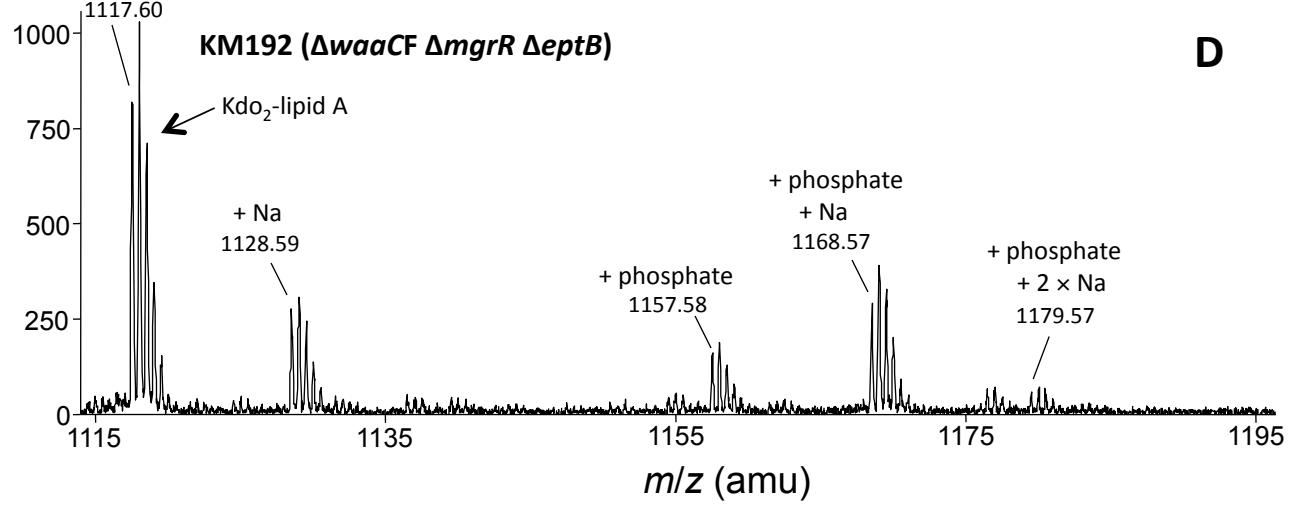
**B**



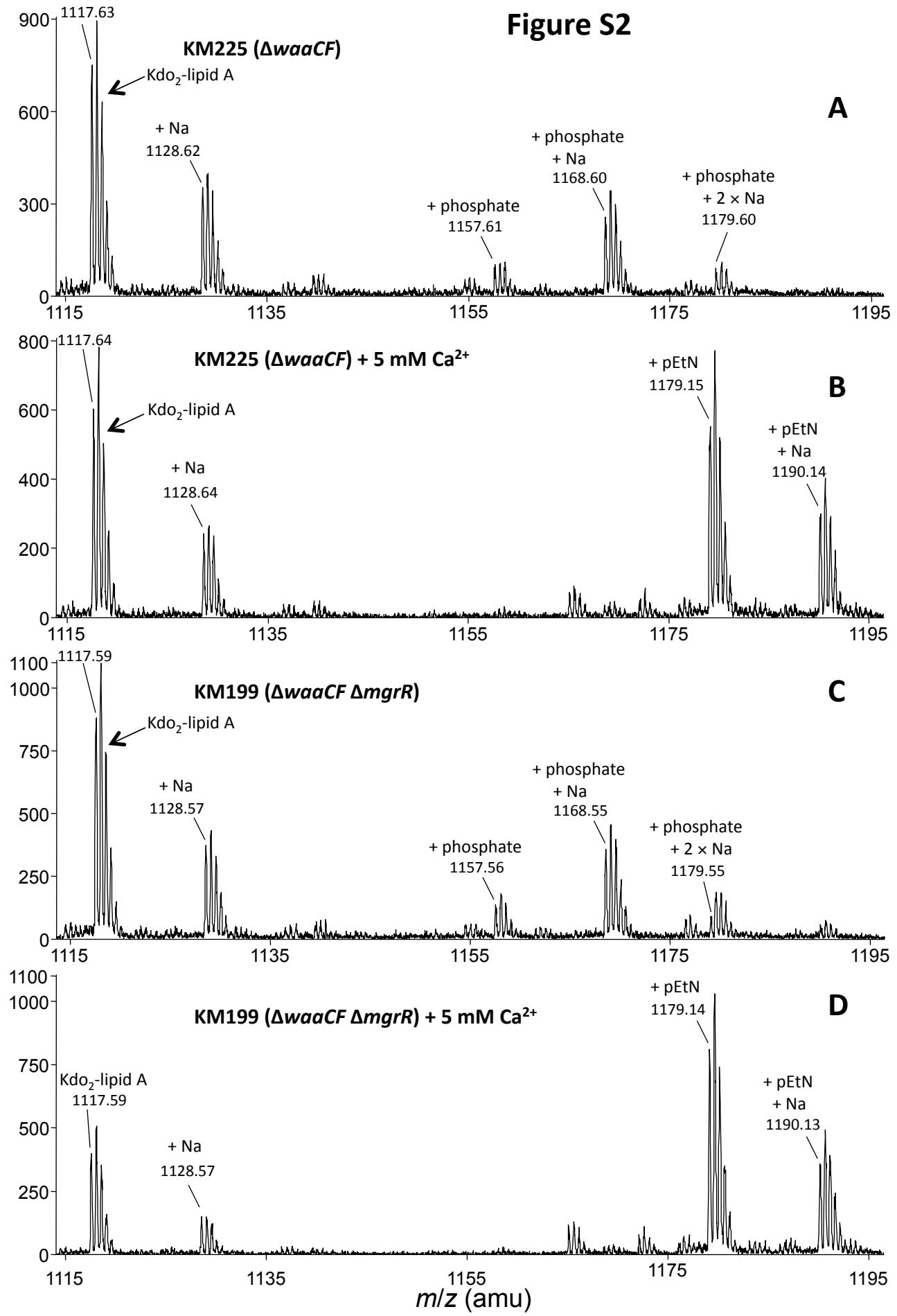
**C**



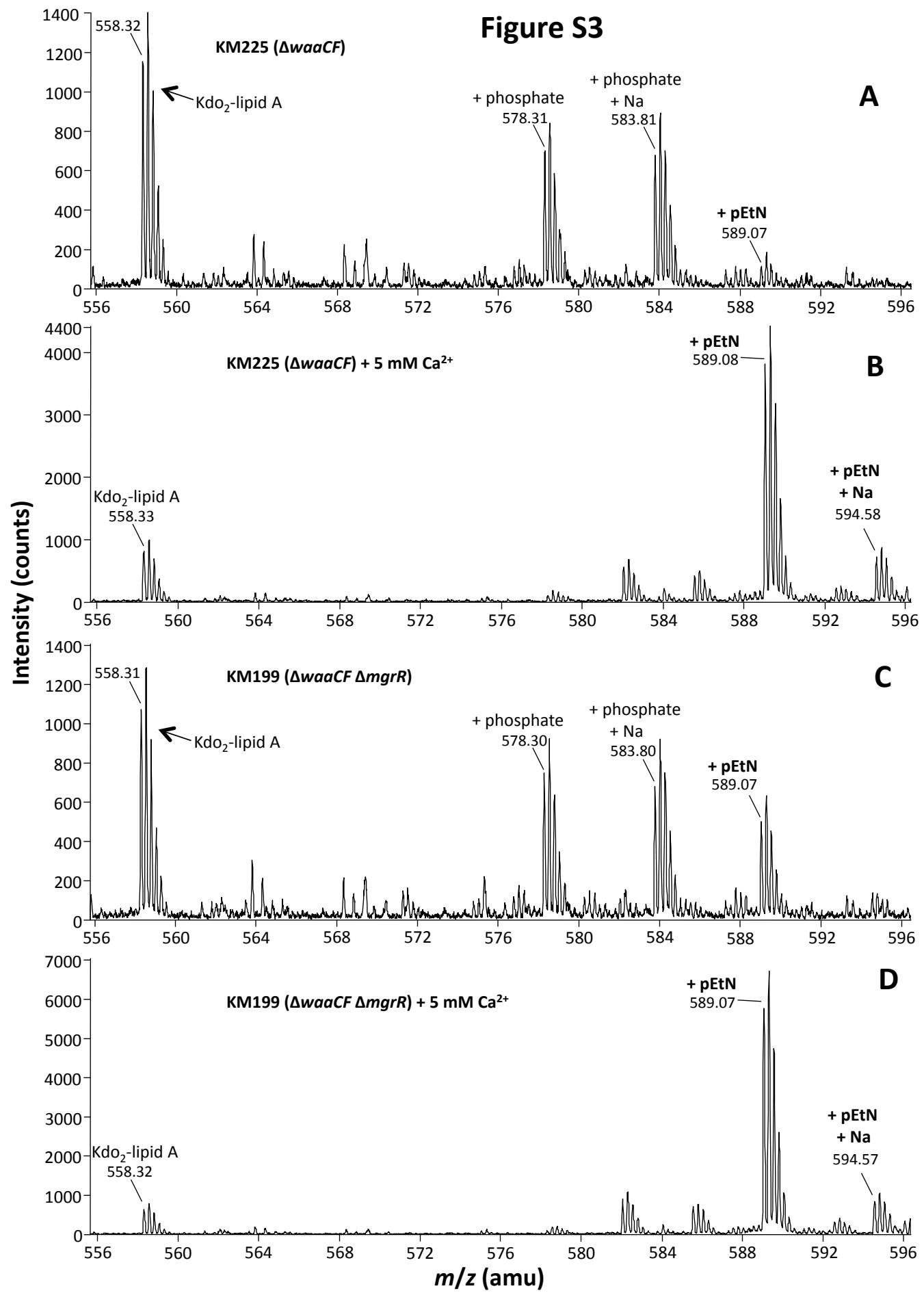
**D**



**Figure S2**



**Figure S3**



**Figure S4: Alignments for the *eptB* promoter and leader**

EcK12	AACGCTAAAGTCTTTCAAACCTGCATTTGTAAATTGCTTCATGCACACTCTT
Efer	AAGTCTCAATTCTACACTTGCGCTTTGTAAATTGCTTCAGACACACGCTT
Salm	tttttgtaaaaatcttccgatcacacttc
EntCl	tcatctcggtcggggcacgtatTTTgtaaatacgtgcTTtat cacattcg
Kleb	agtgc当地ccagccaaatgtttgtaaatgtttggctggatcacattccc
SigE	GGAACCTTT
	TGGTCAAAA 5/6 G/
Eck12	TCCCCACACTTTTCCCTTTGCTGTGGTCTACTTATTCGCGCGTGTAGA-TTTTACTTATC
Efer	TCCAGACACTTTTCTTTAACCTGTGGTCTACTAGCTGCGC-TGTGGA-TTTCATTACTAT
Salm	cgttgc当地ccctctgtgtggctacttaaccgtgctgttagacttcttacatc
EntCl	tgccgctactttccatccccgtggctacttat-cgc当地ctgttagacttctgattaatt
Kleb	tcctgatactttccgttccctgtggctacttat-cgc当地ctgttagaggttctgataac
Extra leader Salm	gcgttagccgcataatttacctgtttctacttaaccgtgctgttagacttcttac
Eck12	TGACTACCTCCGCACTTTCCCTGCCGGGCCTGAAAGCCACTAAGCAGGGTGTATCA
Efer	CGGCAACCTCCCGGTATTCACCTGCCAGGTACATAGATCATCACGCAGGGATTTCA
Salm	atctgc当地tagccgcataatttacctgtttgattaaagaatcggtgacaggcggtttta
EntCl	tataaggcgatattacgc当地catattgc当地ccatggcttttaaccattggctggttttt
Kleb	caaccctaacgc当地ccattagc当地actgactcg当地ccacaggcggtttttat
MgrR:	UUACGAACGUAC-CUAUCU
Eck12	CCTGTTTGTCCAGGGTTTGTGATGAGATACATCAAATCGATTACACAGCAGAAGCTG
Efer	CCTGTTTCCAGGGTTTGTGATGAGATATATCAAACGATAACGCAGCAGCTA
Salm	tccc当地gatccagggtt当地gttgc当地tagagatacatattaaatcgatgacgc当地aaactt
EntCl	tccctttaccagggtt当地gttgc当地tagaaatcgatgacccagcaaaaagctgt
Kleb	tcttctttccagggtt当地gttgc当地tagaaatattagaacgtgacgc当地cagaagctt

**Figure S5**

rpoE P1, P2 transcriptional fusions

